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Effects of ambient particulate matter on aerobic exercise performance

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ABSTRACT

Background/Objective: Wintertime thermal inversions in narrow mountain valleys create a ceiling effect, increasing concentration of small particulate matter (PM_{2.5}). Despite potential health risks, many people continue to exercise outdoors in thermal inversions. This study measured the effects of ambient PM_{2.5} exposure associated with a typical thermal inversion on exercise performance, pulmonary function, and biological markers of inflammation.

Methods: Healthy, active adults (5 males, 11 females) performed two cycle ergometer time trials outdoors in a counterbalanced design: 1) low ambient PM_{2.5} concentrations (<12 µg/m³), and 2) an air quality index (AQI) ranking of “yellow.” Variables of interest were exercise performance, exhaled nitric oxide (eNO), c-reactive protein (CRP), forced vital capacity (FVC), and forced expiratory volume in 1 s (FEV₁).

Results: Despite a significant difference in mean PM_{2.5} concentration of 9.3 ± 3.0 µg/m³ between trials ($p < .001$), there was no significant difference ($p = .424$) in the distance covered during low PM_{2.5} conditions (9.9 ± 1.7 km) compared to high PM_{2.5} conditions (10.1 ± 1.5 km). There were no clinically significant differences across time or between trials for eNO, CRP, FVC, or FEV₁. Additionally, there were no dose-response relationships ($p > .05$) for PM_{2.5} concentration and the measured variables.

Conclusion: An acute bout of vigorous exercise during an AQI of “yellow” did not diminish exercise performance in healthy adults, nor did it have a negative effect on pulmonary function or biological health markers. These variables might not be sensitive to small changes from acute, mild PM_{2.5} exposure.

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Introduction

Wintertime thermal inversions occurring in geographical regions confined by mountains have the potential to trap air pollutants. Due to its geography of a narrow valley bordered by high mountains and frequent winter thermal inversions, Cache Valley in northern Utah is particularly susceptible to episodes of poor air quality.¹ In fact, according to the American Lung Association,² Logan, UT, with a population of about 50,000, ranked number 8 in the United States for short-term particle pollution, just ahead of Los Angeles with a population of about 3.8 million. In January 2004, residents of Cache Valley experienced the nation's worst PM_{2.5} (particulate matter ≤ 2.5 µm in diameter) air pollution episode ever registered.³ The predominant chemical component of the PM in

Cache Valley is ammonium nitrate (NH₄NO₃) formed through acid-base reactions between gas-phase ammonia from the excreta of dairy cattle and nitrogen oxides from vehicle exhaust and other combustion products.³

Previous research has been published specific to the effects of Cache Valley PM on humans. As far back as the mid-1980s data were collected to determine if there was an association between respiratory hospital admissions and PM pollution in the valley.⁴ The results suggested that PM pollution plays a role in the incidence and severity of respiratory disease. More recently, Watterson and colleagues performed a series of studies using cultured human bronchial epithelial cells treated with PM_{2.5} collected in Cache Valley.^{5–7} The PM_{2.5}-exposed cells triggered an inflammatory response, upregulation of cytokine receptors and both interleukins 1 and 6,⁷ through mechanisms involving the unfolded protein response, which is a cellular response to endoplasmic reticulum stress.⁶

It is logical to assume that exposure to ambient PM is likely to increase during bouts of aerobic exercise, placing exercisers at an

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increased health risk. McCafferty⁸ hypothesized three reasons for this increased risk. First, with an increase in V_E during exercise there is an increase in the quantity of pollutants inhaled. Increased PM deposition as a result of the high ventilation rates that occur during exercise has been documented.⁹ Second, a larger percentage of air is inhaled through the mouth during exercise, thereby bypassing some of the filtration that normally takes place in the nasal passages. Third, the velocity of airflow is increased during exercise, and this might carry pollutants deeper into the respiratory tract.

Of the pollutants in the air quality index (AQI) evaluated by the United States Environmental Protection Agency (EPA), PM_{2.5} is the greatest concern in Cache Valley. However, of the limited research on the effects of airborne pollutants on exercisers, the majority of these studies have focused on ozone, and there are very few studies specific to PM.¹⁰ Nevertheless, a review specific to PM and exercise was published by Cutrufello and colleagues.¹¹ This review cited studies that reported a decrease in FVC and FEV₁ among healthy¹² and asthmatic subjects¹³ that exercised near busy streets, and an increase in blood neutrophil concentration following intermittent exercise during wood smoke exposure.¹⁴ However, there was a lack of controlled studies that measured PM levels cited in this review, and no studies specific to PM from thermal inversions.

The purpose of the present study was to measure the effects of ambient PM_{2.5} exposure from a typical thermal inversion in Cache Valley on exercise performance, pulmonary function, and biological markers of inflammation. We hypothesized an exacerbation of the biological damage done by Cache Valley PM during exercise compared to rest, and a decrease in physical performance with exercise in a polluted environment compared to more favorable ambient conditions. Furthermore, a dose-response relationship for pollution and biological markers and performance decrement was hypothesized such that tests performed under the worst air quality would result in the most dramatic changes in biomarkers and produce the greatest challenge to exercise performance.

Methods

Study design and overview

The study used a quasi-experimental within-groups design. Following a preliminary screening session, each study participant performed two trials, separated by at least 48 h. Each trial consisted of a resting portion and an exercise portion. One trial was performed under conditions of low PM_{2.5} concentration (PM_{2.5} of 0–12 $\mu\text{g}/\text{m}^3$), and one trial was done when the air quality was worse but still within the acceptable range established by the EPA (PM_{2.5} of 13–35 $\mu\text{g}/\text{m}^3$). The trials were counterbalanced such that some participants performed their first trial during low PM_{2.5} concentrations while others performed their first trial during higher concentrations.

Participants

Recreationally active adults, defined as exercising at least 150 min/week, were recruited from within Cache Valley, Utah. Those interested in the study completed a preliminary health screening that included a medical history questionnaire, physical examination, and blood chemistry profile. Current smokers, including those who quit less than 6 months prior to the health screening, and those with documented presence of cardiovascular or cardiopulmonary disease were excluded. The study was approved by the IRB of Utah State University (protocol #6117), and participants signed a written informed consent prior to participation.

Procedures

All testing took place in the same location during January and February 2015, as thermal inversions in Cache Valley are most prevalent during winter months. Thermal inversion forecasting was used in an attempt to predict the dates that PM_{2.5} would likely be elevated. Hour-averaged PM_{2.5} data were obtained from the Utah Department of Environmental Quality, Division of Air Quality website (http://www.airquality.utah.gov/aqp/trend_charts/getData.php?id=cache) for each participant during each trial.

Prior to each trial baseline measurements of pulmonary function, exhaled nitric oxide (eNO), and plasma C-reactive protein (CRP) were taken indoors. Pulmonary function (forced vital capacity [FVC] and forced expiratory volume in 1 s [FEV₁]) was assessed with a MicroPlus spirometer (Micro Direct Inc., Lewiston, ME) from a seated position. Fractional eNO, measured by Niox Mino (Circassia Pharmaceuticals Inc., Chicago, IL), is a marker of lung eosinophilic airway inflammation and has been used to monitor the airway inflammatory response to air pollution.¹⁵ Plasma CRP was used as a marker of systemic inflammation. Participants were fitted with a Polar T31 telemetric heart rate (HR) monitor (Polar Electro Oy, Lake Success, NY), and peripheral oxygen saturation (SpO₂) was measured with a finger pulse oximeter (SportStat, Nonin Medical Inc., Plymouth, MN). HR was recorded every 2 min and SpO₂ every 5 min throughout the resting and exercise portions of each trial.

Following baseline measurements, participants went outside for a 20-min seated resting trial, after which FVC, FEV₁, and eNO were repeated. Following this resting period, participants performed a 20-min time trial on a mechanically-braked cycle ergometer (model 824e, Monark Exercise, Vansbro, Sweden) against a resistance that was approximately 3.5% of their body mass. This resistance was determined through pilot testing as being challenging yet a workload that participants could maintain for 20 min. They were instructed to ride as hard as possible for the 20 min, and were blinded to the speedometer and odometer. A time update and verbal encouragement were given at 5 min intervals and during the final 30 s and 10 s. The distance covered was recorded as the measure of performance. Time trials have greater validity and reliability than time to exhaustion tests.¹⁶ Active recovery (slow pedaling against a light load) took place for 5 min then final measurements of FVC, FEV₁, eNO, and CRP were made. Additionally, the participants returned 24 h later for another CRP measurement.

Statistical analyses

Mean comparison of the distance covered between the two 20-min time trials was made with a dependent *t*-test. A two-way repeated-measures ANOVA was used to compare the means of the physiological markers of interest (FVC, FEV₁, eNO, and CRP) across time (baseline, post-rest, and post-exercise) and trials (low PM_{2.5} and high PM_{2.5}). Given that each participant experienced a different range of PM_{2.5} concentrations, regression analysis was done on the PM_{2.5} difference (high PM_{2.5} – low PM_{2.5}) and the magnitude of change in the variables of interest to determine if a dose-response relationship existed. All analyses were done using SPSS (version 24, IBM Corp., Somers, NY). Statistical significance was accepted at an alpha level of $p \leq .05$.

Results

Thirty-three people participated in the preliminary screening process. Two failed the health screening, and five decided not to participate after the initial consultation. Additionally, one subject could not complete the exercise protocol, and one subject

Table 1

Two-way repeated measures ANOVA data (mean with SD in parenthesis) for pulmonary function, exhaled nitric oxide, and C-reactive protein.

| | FVC (L) | | | FEV ₁ (L) | | | eNO (ppb) | | | CRP (mg/L) | | |
|------------------------------|-----------------------|------------------------|-------------------------|------------------------------|------------------------|-------------------------|------------------------------|------------------------|--------------------------|------------------------------|------------------------|----------------------|
| | Low PM _{2.5} | High PM _{2.5} | | Low PM _{2.5} | High PM _{2.5} | | Low PM _{2.5} | High PM _{2.5} | | Low PM _{2.5} | High PM _{2.5} | |
| Baseline | 4.39 (0.99) | 4.50 (0.90) | Time <i>p</i> = .336 | 3.55 (0.88) | 3.70 (0.85) | Time <i>p</i> = .725 | 18.2 (18.8) | 13.6 (8.8) | Time <i>p</i> = .024* | .80 (.63) | .46 (.38) | Time <i>p</i> = .250 |
| Post-rest | 4.32 (1.00) | 4.43 (0.97) | | 3.53 (0.89) | 3.66 (0.86) | | 17.0 (15.1) | 13.0 (7.7) | | .80 (.63) | .47 (.38) | |
| Post-exercise | 4.38 (1.01) | 4.44 (1.05) | | 3.56 (0.92) | 3.63 (0.95) | | 15.1 (15.1) | 12.6 (7.2) | | .67 ^a (.42) | .48 ^a (.43) | |
| Trial <i>p</i> = .071 | | | | Trail <i>p</i> = .035* | | | Trial <i>p</i> = .187 | | | Trial <i>p</i> = .035* | | |
| Trial x Time <i>p</i> = .729 | | | | Trial x Time <i>p</i> = .551 | | | Trial x Time <i>p</i> = .082 | | | Trial x Time <i>p</i> = .121 | | |

FVC = forced vital capacity, FEV₁ = forced expiratory volume in 1 s, eNO = exhaled nitric oxide, CRP = C-reactive protein.^a CRP data were collected at baseline, immediate post-exercise, and 24-h post-exercise.

withdrew due to an injury unrelated to the study. Eight participants never got the opportunity to ride the time trials because anticipated thermal inversions with high concentrations of PM_{2.5} never materialized. Ultimately, 16 participants (5 males, 11 females) completed all aspects of the study. The age and body mass index of the participants were 31.5 ± 11.3 years and 22.6 ± 1.7 kg/m², respectively.

The PM_{2.5} concentrations ranged from a low of 2.1 µg/m³ to a high of 17.7 µg/m³ during testing. Despite a significant difference in mean PM_{2.5} concentration of 9.3 ± 3.0 µg/m³ between trials ($p < .001$), there was no significant difference ($p = .424$) in aerobic performance as measured by the distance covered during low PM_{2.5} conditions (9.9 ± 1.7 km) compared to high PM_{2.5} conditions (10.1 ± 1.5 km). Additionally, the air temperature was not significantly different between trials (4.5 ± 2.7 °C v. 4.5 ± 2.4 °C, $p = .942$).

Data for the two-way repeated measures ANOVA for the health markers are in Table 1. The interaction between time (baseline, post-rest, post-exercise) and trial (low PM_{2.5}, high PM_{2.5}) was not significant for pulmonary function (FVC: $p = .729$; FEV₁: $p = .551$). The main effects of time ($p = .336$) and trial ($p = .071$) were not significant for FVC. The main effect of time was not significant for FEV₁ ($p = .725$), but the main effect of trial was statistically significant ($p = .035$) in the direction opposite of what was expected with the high PM_{2.5} trial resulting in a slightly higher FEV₁ (3.55 ± 0.23 L v. 3.66 ± 0.22 L). However, this statistically significant result of 0.11 L is not likely to be clinically or physiologically significant.

The eNO measurement was significantly ($p = .024$) less post-exercise (13.8 ± 2.9 ppb) compared to post-rest (15.0 ± 3.0 ppb) and baseline (15.9 ± 3.6 ppb), but again these small differences of about 2 ppb have little clinical relevance. The difference between PM_{2.5} trials was not significant ($p = .187$), and neither was the time \times trial interaction ($p = .082$).

Neither the main effect of time ($p = .250$) nor the time \times trial interaction ($p = .121$) was significant for CRP. The main effect of trial was significant for CRP ($p = .035$), with slightly higher values during the low PM_{2.5} condition (0.76 ± 0.15 mg/L) compared to the high PM_{2.5} trial (0.47 ± 0.10 mg/L). Both values are considered “low” for inflammation, and there is no clinically significant difference between them.

The difference in PM_{2.5} concentration between trials of high and low concentration ranged from 5.7 µg/m³ to 15.3 µg/m³. When the difference in concentration between trials was regressed on the variables of interest, there were no significant relationships ($p > .05$) for distance covered, or changes in FVC, FEV₁, eNo, or CRP. This indicates that there were no dose-response relationships for PM_{2.5} concentration and the measured variables.

Discussion

The primary findings from this study were that the exercise performance and biological health markers (FVC, FEV₁, eNO, and CRP) of healthy, fit adults were not negatively affected when

exercising vigorously in ambient PM_{2.5} concentrations that corresponded to an AQI of “yellow” compared to an AQI of “green.” A yellow AQI corresponds to a “moderate” level of health concern for those who are unusually sensitive to air pollution; however, this level of pollution is considered acceptable for the general population. Therefore, our findings support the EPA’s AQI classification that mild air pollution poses no clear negative consequences for healthy adults who are exercising vigorously during yellow AQI warnings.

Previous studies reported an association between PM_{2.5} air pollution and physical inactivity.^{17,18} According to Wen et al.,¹⁹ citizens of counties like Cache Valley in northern Utah, with higher PM_{2.5} pollution are likely to be mindful of AQI announcements and warnings. These warnings, even for moderate pollution, may discourage some individuals to exercise outdoors. There could be the misconception that a yellow AQI is harmful even for healthy individuals who are not unusually sensitive to air pollution. Recently, Tainio and colleagues²⁰ performed a risk-benefit analysis between active travel related to physical activity (cycling and walking) and exposure to air pollution. They examined all-cause mortality for different levels of physical activity and PM_{2.5} values ranging from 5 to 200 µg/m³. For PM_{2.5} values up to 22 µg/m³, the benefits of physical activity far outweigh the risks. Furthermore, they concluded that both the pollution levels and the exercise would have to be extreme for the harms to outweigh the benefits. Combined with the results from the present study, these findings suggest that mild elevations in air pollution are insufficient to result in measureable decrements in acute performance or the health of healthy individuals, even during vigorous exercise.

Nevertheless, several variables must be considered when interpreting these findings and comparing them to previous research. First, the study sample consisted of acclimatized healthy, active adults. The majority of articles cited in a review of particulate matter and exercise¹¹ involved participants with cardiopulmonary disease. In contrast to our study, peak oxygen consumption of cardiac rehabilitation patients decreased by 14.9% per 10 µg/m³ increase in ambient PM_{2.5}, even within acceptable AQI standards,²¹ but we are unaware of any similar studies conducted with mild pollution that have been done on healthy participants.

Second, it is important to note that the winter of 2015 was unusually mild for northern Utah, with warmer than usual temperatures leading to only mild temperature inversions. Consequently, the levels of ambient pollution in our study were substantially lower than those reported in previous studies. For example, Rundell et al.¹² vigorously exercised 12 physically fit males under conditions of low and high pollution, similar to our study design. However, their high pollution trial was conducted next to a major highway and had PM levels that were 34 times greater than their low pollution trial, whereas our participants experienced pollution that was, on average, only 3 times greater. With the large magnitude increase in pollution, Rundell et al.¹² observed a statistically significant decrease in FEV₁, but the

authors noted that this drop was not large enough to be clinically significant.

Third, the easily measured variables of exercise performance and pulmonary function may not be sensitive enough to detect damage from low levels of acute pollution exposure, particularly in participants acclimatized to living in such an environment. Variability of 5% from test to test for FVC and FEV₁ is within the acceptable limits of test-retest reliability,²² and the magnitude of change in pulmonary function following mild exposure to PM_{2.5} may not exceed that threshold. Furthermore, numerous factors other than air pollution can influence exercise performance and pulmonary function, diminishing their value as dependent variables in PM_{2.5} studies. Histological and biochemical analyses would likely be more sensitive to the deleterious effects of low levels of acute PM_{2.5} exposure than exercise performance or pulmonary function. For example, Riva and colleagues²³ demonstrated that even a single low-dose exposure of PM_{2.5} induced lung tissue inflammation and oxidative stress in mice.

There is a lack of research examining the impact of mild air pollution on vigorous exercise in healthy people. Furthermore, to our knowledge, this is the first study to examine the acute effects of mild ambient PM_{2.5} concentrations (“yellow” AQI) associated with a thermal inversion on exercise and pulmonary function in healthy exercisers. For researchers attempting to conduct similar field studies of exercise in polluted thermal inversions, we offer the following observations. This type of research involves many logistical challenges. It is possible to obtain forecasts of thermal inversions; however, like all weather phenomena, inversion forecasting is not always accurate. Additionally, although PM_{2.5} concentration typically increases during a thermal inversion, the strength and duration of the inversion as well as other non-meteorological factors such as pollutants from automobiles or heating sources will influence the magnitude of the PM_{2.5} pollution. Due to rapidly changing weather patterns, study participants and the research team need to be available on short notice. Finally, even with an inversion present, ambient PM_{2.5} concentration varies throughout the day just like temperature and wind speed; thus, PM_{2.5} concentration should be measured regularly while testing.

Conclusion

The main finding from this study was that an acute bout of vigorous exercise in ambient PM_{2.5} pollution equivalent to an AQI of “yellow” did not diminish the exercise performance of healthy, avid exercisers, nor did it have a negative effect on pulmonary function testing or biological health markers. Exercise performance and pulmonary function testing of healthy adults may not be sensitive enough to detect small deleterious effects that likely occur from acute exposure to mild ambient PM_{2.5} pollution.

Conflicts of interest statement

The authors have no conflicts of interest relevant to this article.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.jesf.2018.01.002>.

References

1. Silva PJ, Vawdrey EL, Corbett M, et al. Fine particle concentrations and composition during wintertime inversions in Logan, Utah, USA. *Atmos Environ*. 2007;41:5410–5422.
2. American Lung Association. *Most Polluted Cities*. American Lung Association web site; 2017. <http://www.lung.org/our-initiatives/healthy-air/sota/city-rankings/most-polluted-cities.html>. Accessed August 19, 2017.
3. Malek E, Davis T, Martin RS, et al. Meteorological and environmental aspects of one of the worst national air pollution episodes (January, 2004) in Logan, Cache Valley, Utah, USA. *Atmos Res*. 2006;79:108–122.
4. Pope CA. Respiratory hospital admissions associated with PM10 pollution in Utah, Salt lake, and cache valleys. *Arch Environ Health*. 1991;46:90–97.
5. Watterson TL, Hamilton B, Martin R, et al. Urban particulate matter activates Akt in human lung cells. *Arch Toxicol*. 2012;86:121–135.
6. Watterson TL, Hamilton B, Martin R, et al. Urban particulate matter causes ER stress and the unfolding protein response in human lung cells. *Toxicol Sci*. 2009;112:111–122.
7. Watterson TL, Sorensen J, Martin R, et al. Effects of PM2.5 collected from Cache Valley Utah on genes associated with the inflammatory response in human lung cells. *J Toxicol Environ Health*. 2007;70:1731–1744.
8. McCafferty WB. *Air Pollution and Athletic Performance*. Charles C. Thomas: Springfield; 1981.
9. Daigle CC, Chalupa DC, Gibb FR, et al. Ultrafine particle deposition in humans during rest and exercise. *Inhal Toxicol*. 2003;15:539–552.
10. Cheung SS. *Advanced Environmental Exercise Physiology*. Champaign, IL: Human Kinetics; 2010:191–206.
11. Cutrufello PT, Smoliga JM, Rundell KW. Small things make a big difference: particulate matter and exercise. *Sports Med*. 2012;42:1041–1058.
12. Rundell KW, Slee JB, Caviston R, et al. Decreased lung function after inhalation of ultrafine and fine particulate matter during exercise is related to decreased total nitrate in exhaled breath condensate. *Inhal Toxicol*. 2008;20:1–9.
13. McCreanor J, Cullinan P, Nieuwenhuijsen MJ, et al. Respiratory effects of exposure to diesel traffic in persons with asthma. *N Engl J Med*. 2007;357:2348–2358.
14. Ghio AJ, Soukup JM, Case M, et al. Exposure to wood smoke particles produces inflammation in healthy volunteers. *Occup Environ Med*. 2012;69:170–175.
15. Zuurbier M, Hoek G, Oldenwening M, et al. Respiratory effects of commuters' exposure to air pollution in traffic. *Epidemiology*. 2011;22:219–227.
16. Currell K, Jeukendrup AE. Validity, reliability and sensitivity of measures of sporting performance. *Sports Med*. 2008;38:297–316.
17. Roberts JD, Voss JD, Knight B. The association of ambient air pollution and physical inactivity in the United States. *Plos One*. 2014;9(3), e90143.
18. Wen X, Balluz LS, Shire JD, et al. Association of self-reported leisure-time physical inactivity with particulate matter 2.5 air pollution. *J Environ Health*. 2009;72:40–44.
19. Wen X-J, Balluz L, Mokdad A. Association between media alerts of air quality index and change of outdoor activity among adult asthma in six states, BRFSS. *J Community Health*. 2005;2009(34):40–46.
20. Tainio MK, de Nazelle AJ, Gotschi T, et al. Can air pollution negate the health benefits of cycling and walking? *Prev Med*. 2016;187:233–236.
21. Giorgini P, Rubenfire M, Das R, et al. Higher fine particulate matter and temperature levels impair exercise capacity in cardiac patients. *Heart*. 2015;101:1293–1301.
22. American Thoracic Society. Standardization of spirometry: 1994 update. *Am J Respir Crit Care Med*. 1994;152:1107–1136.
23. Riva DR, Magalhaes CB, Lopes AA, et al. Low dose of fine particulate matter (PM2.5) can induce acute oxidative stress, inflammation and pulmonary impairment in healthy mice. *Inhal Toxicol*. 2011;23:257–267.